Conservation Genet Resour (2009) 1:179–181
DOI 10.1007/s12686-009-9044-5

TECHNICAL NOTE

Characterization of microsatellite loci in *Leucorrhinia caudalis*, a rare dragonfly endangered throughout Europe

Daniela Keller · Sabine Brodbeck · Rolf Holderegger

Received: 12 June 2009 / Accepted: 14 June 2009 / Published online: 1 July 2009

Abstract *Leucorrhinia caudalis* is a rare dragonfly, threatened throughout Europe. It only survived in a single population in Switzerland in the 1980s. However, it recently spread and colonized new ponds. In order to be able to study contemporary migration in this species, eight new microsatellite markers were developed and tested on 24 individuals from six Swiss ponds. We detected three to eleven alleles per polymorphic locus and found observed and expected heterozygosities of 0.250 to 0.875 and 0.215 to 0.840, respectively.

Keywords Conservation · Dragonfly · *Leucorrhinia caudalis* · Libellulidae · Microsatellites

Leucorrhinia caudalis (Charpentier, 1840; Libellulidae) is a rare dragonfly threatened throughout its whole European distribution area, which ranges from France to southern Scandinavia and to western Siberia. In Switzerland, the species was once widespread, but persisted in only a single population in 1980. However, colonisations of new ponds were recorded in the 1990s and 2000s (Vonwil 2005). Currently, there are six larger sites of *L. caudalis* in Switzerland. In order to establish contemporary migration among these Swiss populations by means of assignment tests (Cornuet et al. 1999) and thereby testing whether structural connectivity measures provide functional connectivity (Holderegger and Wagner 2008), we developed microsatellite markers for *L. caudalis* as no such markers were available for this species so far.

D. Keller (✉) · S. Brodbeck · R. Holderegger
WSL Swiss Federal Research Institute, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland
e-mail: daniela.keller@wsl.ch

The development of microsatellite markers was carried out by ecogenics GmbH (Zurich, Switzerland). An enriched library from size selected genomic DNA was established from a larva of *L. caudalis*, ligated into SAULA/SAULB-linker (Armour et al. 1994) and enriched by magnetic bead selection with biotin labelled $(GT)_{13}$, $(CT)_{13}$, $(AAC)_{10}$ and $(AAG)_{10}$ oligonucleotide repeats (Gautschi et al. 2000a, b). From the 750 recombinant colonies screened, 64 positive clones were sequenced, and primers were designed for 13 microsatellite inserts. After PCR optimization, five microsatellite regions could be amplified reliably. In a second step, plasmids from another 50 positive clones were sequenced and primers for another eight microsatellite inserts were designed, of which three could be amplified reliably. Finally, PCR amplifications were performed for eight microsatellite regions.

These eight microsatellites were tested on 24 individuals. A non-invasive sampling strategy was used, in order to not affect the populations of the threatened *L. caudalis*. Genomic DNA was isolated from exuviae (Watts et al. 2005). Exuviae were collected at six ponds in Switzerland and stored in 100% ethanol at room temperature in the dark. Before DNA extraction, exuviae were placed in a sterile bench in order to evaporate any ethanol. After drying and removing the head part, exuviae were put into Eppendorf tubes with steel beads and ground with a MM300 mixer mill for 3 min (Retsch). Extraction was carried out using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturers instructions with the two following modifications. Proteinase K incubation was for 3 h and the elution step was performed twice with 50 µl AE buffer. PCR was carried out in 10 µl reaction volumes containing 5 µl 2× Multiplex Mix (Qiagen), 0.2 µM of each primer, 2 ng of diluted genomic DNA and ddH_2O. Amplifications were run on a Veriti thermal cycler (Applied Biosystems), using the

Table 1 Characteristics of eight microsatellite DNA loci developed for *Leucorrhinia caudalis* and tested for a total of 24 individuals collected from six ponds in Switzerland

Locus	Primer sequences (5′–3′)	Repeat motif	Annealing temperature (°C)	N_A	H_o	H_e	Size range (bp)	HWE test	GenBank accession number
Leucau03	F: TGGGTGAGAATATAAAAGCAAGG	$(GA)_5TT(GA)_{24}$	56	11	0.750	0.711	76–142	0.754	FJ976713
	R: GCGGAAACGAAAAGCTGTAG								
Leucau04	F: AGAAGGGCAAGCAGTAATGG	$(TG)_{14}TT(TC)_6$	56	3	0.250	0.215	86–98	0.999	FJ976714
	R: CTCAGCGCAGAACGTGTTTC								
Leucau06	F: GTTCTGCCTGTTGGCAAGTC	$(CT)_5TCTT(CT)_6(TC)_{12}$	56	11	0.875	0.840	184–264	0.758	FJ976718
	R: GGAAAGCTGAAATTGATAGAATCAG								
Leucau08	F: CGCATGTATTCCTGGATGAG	$(TTG)_8(GTG)_4$	58	8	0.750	0.561	124–154	0.999	FJ976715
	R: CATCGTGGTAGTGGATGGTG								
Leucau09	F: GTTCGGCTTCGGTGAAAG	$(AAC)_7$	56	1	0.000	–	165	–	FJ976716
	R: CTTAGTCGGAAAAGCGTTCG								
Leucau10	F: AGTCGGCAGATAAAAGGGAAG	$(GA)_{17}$	58	6	0.625	0.778	211–227	0.266	FJ976717
	R: GGCATGAAACACCATGTACG								
Leucau15	F: GACAGAGTGCCTGAATGTGC	$(TC)_{25}$	56	10	0.729	0.833	166–206	0.387	FJ976719
	R: ATCTTCCGCGTAAGCTCTTG								
Leucau20	F: TCCAGCATTTTGAACAAAGAAC	$(CA)_{17}$	56	5	0.375	0.472	209–229	0.221	FJ976720
	R: CGCGACTTTCTGTAGGGAAG								

Primer sequence (F: forward; R: reverse); sequence motif; annealing temperature; N_A = number of observed alleles; H_o = observed heterozygosity; H_e = expected heterozygosity; allele size range; *P*-value of test for Hardy–Weinberg equilibrium (HWE); GenBank accession number

following PCR protocol (ramp 1.2°C/s): 15 min of polymerase activation at 95°C, followed by 35 cycles of denaturation for 30 s at 95°C, 30 s at annealing temperature (varying per primer pair, Table 1), 60 s extension at 72°C and a final elongation of 30 min at 72°C. As non-invasive sampling often encounters difficulties during PCR-amplification (Taberlet and Luikart 1999), each DNA sample was amplified twice. Fragments were analysed on an ABI 3130 sequencer (Applied Biosystems), and genotypes scored with GENEMAPPER 3.7 (Applied Biosystems). One primer (Leucau08) proved to be difficult to score, while the other seven markers were easy to analyse.

FSTAT 2.9.3.2 (Goudet 1995) was used to determine the number of alleles per locus, observed and expected heterozygosity (H_o and H_e) and to test for linkage disequilibrium and Hardy–Weinberg equilibrium (Table 1). One marker (Leucau09) was monomorphic across the small sampling area of the present study, but this locus might well be polymorphic when studying a larger area. Three to eleven alleles were found per polymorphic locus. Observed heterozygosity H_o ranged from 0.250 to 0.875, and H_e varied between 0.215 and 0.840 per locus (Table 1). There was no linkage disequilibrium among all pairs of loci (at $\alpha = 0.05$) and there was also no evidence for a deviation from Hardy–Weinberg equilibrium at any locus (at $\alpha = 0.05$; Table 1).

The microsatellites presented here will serve as genetic markers for an investigation of contemporary migration in *L. caudalis*. They can also form the basis for further investigations concerning the conservation management of this endangered species.

Acknowledgements We thank all the people who collected exuviae, Isabelle Flöss and Gerhard Vonwil for their support and the Canton of Aargau for financial support.

References

Armour JAL, Neumann R, Gobert S, Jeffreys AJ (1994) Isolation of human simple repeat loci by hybridization selection. Hum Mol Genet 3:599–605

Cornuet JM, Piry S, Luikart G, Estoup A, Solignac M (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153:1989–2000

Gautschi B, Tenzer I, Muller JP, Schmid B (2000a) Isolation and characterization of microsatellite loci in the bearded vulture (*Gypaetus barbatus*) and cross-amplification in three old world vulture species. Mol Ecol 9:2193–2195

Gautschi B, Widmer A, Koella J (2000b) Isolation and characterization of microsatellite loci in the dice snake (*Natrix tessellata*). Mol Ecol 9:2191–2193

Goudet J (1995) FSTAT (Version 1.2): a computer program to calculate F-statistics. J Hered 86:485–486

Holderegger R, Wagner HH (2008) Landscape genetics. BioScience 58:199–207. doi:10.1641/B580306

Taberlet P, Luikart G (1999) Non-invasive genetic sampling and individual identification. Biol J Linn Soc 68:41–55

Vonwil G (2005) Leucorrhinia caudalis. Fauna Helv 12:294–298

Watts PC, Thompson DJ, Daguet C, Kemp SJ (2005) Exuviae as a reliable source of DNA for population-genetic analysis of odonates. Odonatologica 34:183–187